

Vaginal antibody of patients with trichomoniasis is to a prominent surface immunogen of *Trichomonas vaginalis*

J F Alderete, E Newton, C Dennis, J Engbring, K A Neale

Abstract

Twenty vaginal washes (VWs) and ten vaginal mucus (VM) samples from patients with trichomoniasis were examined for the presence of antibody to surface protein immunogens of *Trichomonas vaginalis*. Fourteen of 20 VWs (70%) and 8 of 10 VM (80%) had immunoglobulin G (IgG) antibody (Ab) that reacted in an immunoprecipitation (IP) assay with one iodinated *Trichomonas vaginalis* surface protein immunogen with a relative molecular mass of 230,000 daltons (230-kDa) (P230). No similar IP of any iodinated protein was observed when detergent extract was first depleted of P230 with monoclonal antibody (MAb), indicating a highly specific VW IgG response of patients to P230. VWs were also obtained from 10 patients from one to four weeks after treatment. These VWs had the same, or in one case a greater, level of IgG to P230. Under no circumstances was Ab to P230 or any other trichomonad protein detected in VWs or VM from normal, uninfected women. Flow cytometry with VW Ab yielded heterogeneous fluorescent and non-fluorescent populations of trichomonads, reaffirming the restricted Ab response to one or a few epitopes on P230 in the vagina of patients. Under identical conditions, the MAb gave totally fluorescent parasite populations of some isolates, and the MAb again demonstrated variable epitope accessibility to Ab binding (*Infect Immun* 1987;55:1037). Finally, the MAb or VW Ab was never cytolytic for immunoreactive (fluorescent) parasites, even in the presence of complement. This study identifies the most important trichomonad surface immunogen on the basis of the vaginal Ab response, and data underscore the significance of immune evasion strategies of this sexually transmitted disease agent.

Departments of Obstetrics and Gynecology, and Microbiology, The University of Texas Health Science Center, San Antonio, Texas
J F Alderete, E Newton, C Dennis, J Engbring, K A Neale

Introduction

Trichomonas vaginalis causes a world-wide sexually transmitted disease that affects women.¹ The infection is non-self-limiting and incurs a significant emotional and economic burden in patients with trichomoniasis. No evidence is available on any host immunity towards this microorganism, even after repeated infections and in spite of the presence of vaginal wash (VW) immunoglobulin G (IgG) and IgA reactive with trichomonad surface immunogens.²⁻⁴ At this time, however, nothing is known about the identity of any trichomonad surface immunogen(s) detected by antibodies present in the vagina of patients.

This report describes for the first time the specific Ab response in the VW of patients toward a surface protein of *T. vaginalis*. The vaginal Ab response appears highly restricted and does not detect all live trichomonads of infecting isolates. The VW Ab immunoreactive with live parasites was not cytolytic, even in the presence of complement. Antibody to this trichomonad immunogen was still present up to four weeks after drug treatment of patients.⁵ These data further establish the immune evasion capabilities of this pathogen, and the knowledge gained from these studies may ultimately allow us to understand the survival of the parasite even in the face of a host antibody response at the site of infection. With this knowledge strategies to overcome the parasite's immune evasion capabilities or alternative approaches might be devised to control this protozoan parasite.

Materials and methods

Parasites and radiolabelling

Isolate NYH 286 and fresh clinical isolates, such as T032 and T040, were used for experiments and were obtained as previously described.²⁻⁶⁻¹⁰ Trichomonads were passaged daily in a complex medium of trypticase-yeast extract-maltose supplemented with heat-inactivated horse serum.¹¹

Surface radioiodination of washed, live organisms was performed using the chloramine-T iodination procedure, which has been described previously for efficient labelling of trichomonad surface proteins.¹⁰

Intrinsic labelling with [³⁵S]methionine was also as previously detailed¹⁰ for preparation of detergent extracts for immunoprecipitation (IP) experiments as described below.

Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE)

Electrophoresis using 7.5% separating acrylamide gels and electrophoretic conditions were as recently described.¹⁰

Vaginal wash (VW) and vaginal mucus (VM)

VW was obtained using the procedure for isolation of vaginal epithelial cells (VECs),⁶ except that following low speed centrifugation of VECs, the VW was re-centrifuged at 17 500 *g* for 10 minutes to remove any remaining debris and bacteria. Finally, VW was passed through a 0.45 μ m filter. Where needed, VWs of patients having Ab to the trichomonad immunogen were pooled or individually concentrated 10-fold by a rotary evaporator. VWs from normal, uninfected women were also processed similarly.

VM samples (~0.2 ml) recovered from patients using sterile tuberculin syringes with attached small diameter rubber tubing were mixed with 0.5 ml phosphate-buffered saline (PBS) containing standard penicillin-streptomycin for tissue culture. After overnight incubation at room temperature, the mucus was vortexed, and the insoluble mucus was removed by centrifugation. The PBS (~0.5 ml) containing soluble material, referred to as VM extract, was immediately filtered. This VM extract (0.4 ml) was then divided into two equal aliquots, and each aliquot was added to individual microfuge tubes containing a detergent preparation of radioiodinated or ³⁵S-labelled trichomonads of isolate NYH 286, the common laboratory isolate used extensively for surface immunochemical characterisation studies and known to contain the various prominent immunogens of *T. vaginalis*.²⁻⁶⁻¹⁰ The remaining 0.1 ml VM extract was pooled for use in a separate experiment described below.

IP assay

One hundred μ l of 10% (w/v) fixed *S. aureus* washed and suspended in 0.05% Zwittergent 3-12 (Z3-12) (Calbiochem-Behring, La Jolla, Ca) in PBS was incubated with an equal volume of 1:200 dilution of the IgG fraction of goat anti-human IgG (Bio Rad Labs, Richmond, CA) for up to two hours at 4°C. Alternatively, *S. aureus* was pretreated with IgG fraction of rabbit anti-human total Ig (Bio Rad). This washed, pretreated bacterial preparation was then incubated with VW. A 100 μ l volume of this *S. aureus* suspension was then added to 200 μ l of a detergent

preparation of radiolabelled *T. vaginalis*.⁷⁻¹⁰ For the trichomonad preparation, ~2 \times 10⁶ radiolabelled trichomonads were solubilised in 1 ml of 0.05% final concentration prepared in PBS.¹⁰ Bacteria alone or pretreated with anti-human antibodies were also added to the trichomonad detergent preparation as controls. After extensive washing, the bacteria-antibody-antigen complex was boiled for 3 minutes in dissolving buffer for SDS-PAGE and subsequent autoradiographic detection of immunoprecipitated trichomonad proteins.¹⁰ All IP experiments were performed at least three times for VWs and twice for VM.

IP experiments were also performed using a MAb of isotype IgG_{2b} called DM126⁷ that reacts with a M_r 230-kDa (P230) trichomonad surface protein. This protein was distinguished from other surface proteins in IP experiments with MAbs that recognise a M_r ~270-kDa protein (P270) (MAb C20A3, isotype IgG_{2a}) and a M_r ~65-kDa protein (P65) (MAb C55, isotype IgG₁), as previously reported.⁷⁻¹⁰ Irrelevant MAbs were also employed as controls in order to show specificity in the assays.⁷⁻⁸

In separate IP experiments the DM126 MAb-reactive immunogen was first depleted from the trichomonad detergent preparation by adsorption with *S. aureus* pretreated with DM126.⁷⁻¹⁰ This P230-adsorbed detergent preparation, which still possessed P270 and P65 detectable with C20A3 and C55 MAbs, respectively, was used in the IP assay to show the specific anti-P230 response in the patient vaginal secretions.

Flow cytometry and cytotoxicity of live parasites with MAB and VW antibody

Indirect immunofluorescence monitored by flow cytometry as described previously⁷⁻¹⁰ was performed with live trichomonads treated with MAb DM126 or VWs. Experiments were also done to measure the cytolytic ability of the MAb DM126 of VW Ab, and in this case the release of [³H]thymidine-labelled DNA of live organisms was monitored as previously documented.⁸ Irrelevant MAbs of the same isotype but without reaction with live organisms served as negative controls.

Results

The identity of the trichomonad surface protein recognised by vaginal Ab

Figure 1 shows the reaction of Ab of five VM samples with a M_r 230-kDa immunogen, called P230, of *T. vaginalis*. An intense band was seen in eight of 10 VM samples tested, and representative reactions based on iodinated protein band intensities are shown in lanes 2 through 5. Mucus, from control, uninfected individuals, did not have any reactivity with radio-

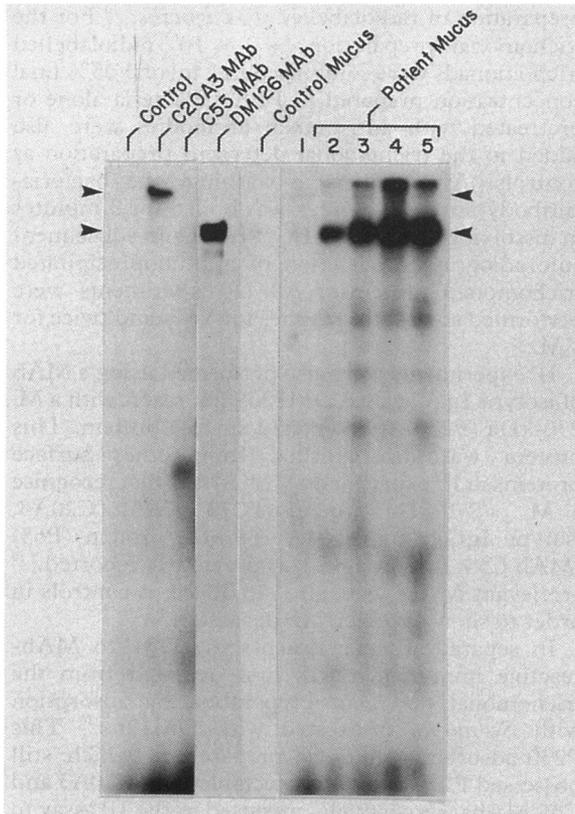


Fig 1 Immunoreactivity of vaginal mucus antibody with surface proteins of *T vaginalis*. Procedures for extracting antibody from mucus and for performing the immunoprecipitation (IP) assay with a detergent preparation of iodinated trichomonad surface proteins are as described in Materials and Methods. For these experiments *S aureus* bacteria were pretreated with IgG fraction of goat anti-human IgG for adsorbing antibody in vaginal mucus extract. Control mucus refers to an IP reaction done identically but with mucus from a normal, uninfected human. The monoclonal antibodies (MAb) are also as described in Materials and Methods. Control refers to the specific reaction using an irrelevant MAb of isotype IgG2b that does not react with trichomonad surface proteins but which has the same isotype as DM126. A similar absence of reactivity by irrelevant MAbs with isotypes of the other MAbs (C20A3 and C55) was obtained.

iodinated trichomonad proteins in the detergent preparation.

A MAb called DM126 reacted with an iodinated surface protein having the same M_r as the major band obtained with VM (fig 1). Therefore, experiments were also performed with a P230-depleted detergent preparation of *T. vaginalis*. The P230-depleted detergent preparation used in the IP assay on duplicate VM samples gave no detectable P230 band, like that seen for lane 1, for example. On the other hand the other MAbs still immunoprecipitated P270 and P65 surface proteins as seen on the autoradiograms labelled C20A3 MAb and C55 Mab,

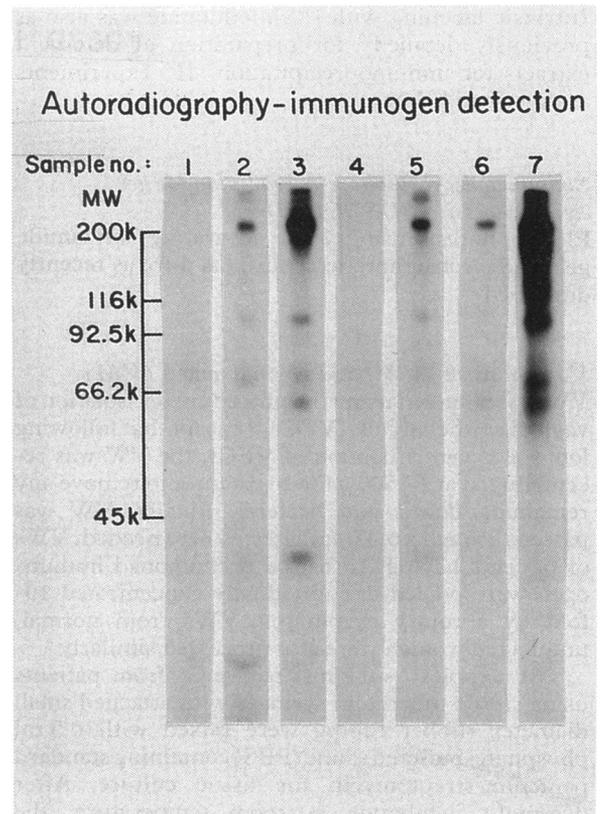


Fig 2 Autoradiogram patterns showing the IP by antibody in vaginal wash (VW) of a surface protein of *T vaginalis*. *S aureus* were first pretreated with IgG fraction of goat anti-human IgG before incubating with VW. The use of VW from normal, uninfected women in the IP assay gave similar absence of reactivity as did some patient VWs, like that seen for samples number 1 and 4, for example.

respectively. These data show the highly specific nature of the vaginal Ab response primarily to this trichomonad surface protein.

Experiments were similarly performed with VW Ab. An ~230-kDa iodinated protein was again immunoprecipitated with VW (fig 2). Only six of 20 VWs (30%) did not react with this immunogen, while the remaining VWs (70%) gave a well-defined and intense protein band in autoradiograms, indicative of Ab to P230. An exception can be seen in the autoradiogram of patient no. 7, which had numerous iodinated proteins. Finally, VW from uninfected women did not react with any trichomonad proteins, again showing the specificity of the assay. Furthermore, as controls, *S. aureus* alone or first pretreated with the anti-human IgG failed to precipitate any iodinated trichomonad proteins, and the autoradiograms were always without protein bands.

The use of a P230-adsorbed trichomonad detergent preparation with VW, including the VW of patient

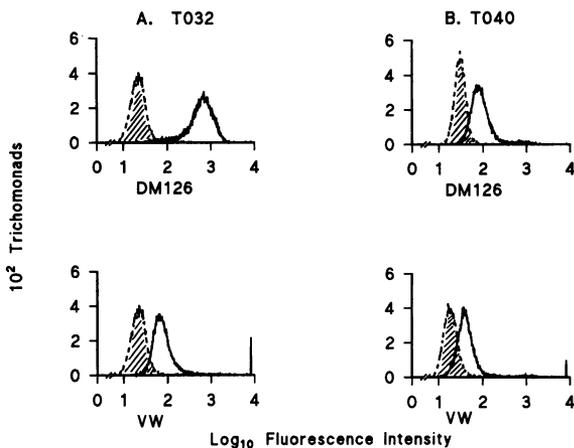


Fig 3 Flow cytometry patterns of two fresh isolates showing the VW Ab immunoreactivity with live trichomonads. Isolate T040 is representative of those which have been defined as Type II on the basis of phenotypic variation for the surface expression of the P270 immunogen as described in Materials and Methods and analysed in fig 1.⁷ Isolate T032 is defined as Type I, indicating an absence to undergo phenotypic variation for the P270 immunogen.⁷ These Type I isolates remain non-fluorescent during flow cytometric analysis for MAb C20A3 reactivity. Note the different fluorescent patterns as seen by the DM126 MAb in contrast to VW Ab, which gave heterogeneous fluorescent (solid lines) and non-fluorescent (lined areas) trichomonads. The dashed lines refer to the control reaction of parasites with irrelevant MAb for the DM126 experiment and with VW Ab from normal, uninfected women for the patient VW Ab experiment, respectively.

no. 7, failed to give any bands on the autoradiograms, indicating that P230 was the prominent immunogen seen by VW Ab. The absence of minor bands for patient 7 VW when the P230-depleted preparation was used may be indicative of proteinase degradation of P230. This is likely, since *T. vaginalis* cells and extracts have been shown to possess many cysteine proteinases,^{12,13} which degrade trichomonad proteins following solubilisation in the detergents used for the IP assays.⁹

As an alternative to pretreating *S. aureus* with anti-human IgG, the protein A-bearing staphylococci were also incubated with anti-human Ig, followed by addition of VW for binding of human Ab. Identical results were obtained, indicating that IgG in VW was the primary immunoglobulin type directed toward P230. Using anti-human Ig as the capture Ab did not give any additional reactivity or increase in band intensity during IP assays.

The surface nature of P230 has been established⁷ and was reaffirmed in this study by experiments showing the removal of the band from autoradiograms when iodinated trichomonads were first trypsinised before solubilization for the IP assay. Finally, the parasite origin of the immunogen detec-

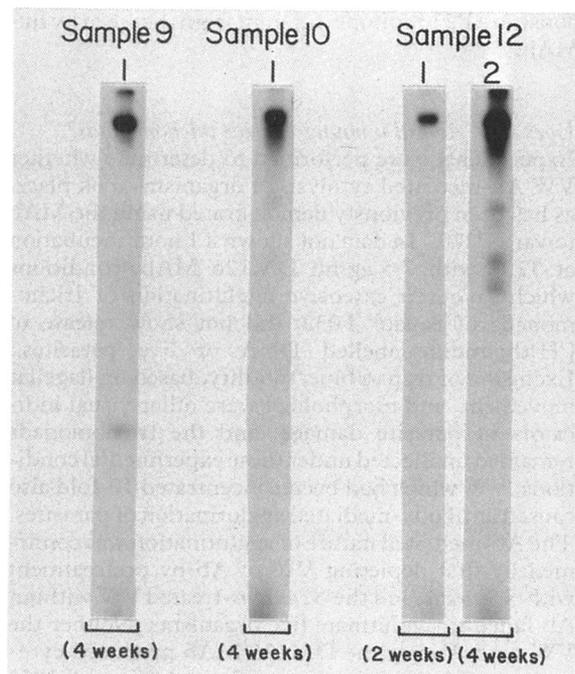


Fig 4 Demonstration by IP of the prolonged nature of the VW Ab response toward P230 after treatment and cure of patients. The time after treatment when VW was obtained is indicated below each IP reaction.

ted by VW antibody was also confirmed previously⁷ by showing that MAb DM126 reacted with a P230 protein from extracts of [³⁵S]-labelled parasites.

VW Ab shows heterogeneous populations by flow cytometry

Figure 3 shows patterns of fluorescence of duplicate preparations of trichomonads of two representative fresh isolates incubated with the DM126 MAb⁷ and with pooled VW of patients. Isolate T032 was representative of a homogeneous immunoreactive population of fluorescent organisms with MAb DM126. Isolate T040, on the other hand, when probed with the MAb, showed subpopulations of both fluorescent and non-fluorescent organisms. The surface expression but MAb inaccessibility to epitope binding has been shown previously for the MAb-nonfluorescent parasites.⁷ Furthermore, changes in the ratio of fluorescent to nonfluorescent organisms seen during daily in vitro passage of the parasites was indicative of epitope phenotypic variation described earlier.⁷

Surprisingly, pooled VWs containing Ab to P230 detected only some, but not all, parasites in both isolates. The fact that VW antibody detected only a subpopulation in isolate T032, in contrast to the DM126 MAb, suggests a restricted antibody res-

ponse to a P230 epitope different from that seen by the MAb.

Does VW Ab kill immunoreactive trichomonads?

Experiments were performed to determine whether VW Ab-mediated cytolysis of organisms took place, as has been previously demonstrated using the MAb toward P270.⁸ In data not shown a 1 hour incubation at 37°C with 7.5 µg/ml DM126 MAb, conditions which favoured extensive agglutination of trichomonads of isolate T032, did not show release of [³H]thymidine-labelled DNA of live parasites.⁸ Exclusion of trypan blue, motility, based on flagellar movement, and morphology were other visual indicators of parasite damage, and the trichomonads remained unaffected under these experimental conditions. VW which had been concentrated 10-fold also caused antibody-mediated agglutination of parasites. The Ab-mediated nature of agglutination was confirmed by first depleting VW of Ab by pretreatment with *S. aureus*, and the *S. aureus*-treated VW without Ab failed to agglutinate live organisms. Neither the VW with Ab nor the DM126 MAb produced cytotoxicity of organisms, even after addition of 10% guinea pig serum as a source of complement.

Ab to P230 does not immediately disappear following treatment of patients

Figure 4 shows the autoradiograms from the IP assay performed with VWs of four patients re-examined at later dates, as indicated. These VWs still had Ab to P230 following treatment. It was possible to obtain VW on more than one occasion after treatment, and this individual had no evidence of a *T. vaginalis* infection at this time based on absence of parasites by microscopic and culture detection. The patient VWs still possessed Ab, and in the case of one patient (sample 12), VW obtained at 4 weeks possessed higher levels of reactivity to P230 than VW at 2 weeks. The reactivity was again demonstrated to be directed to only P230 by experiments using P230-adsorbed trichomonal detergent preparations, as previously mentioned.

Discussion

The presence of vaginal IgG Ab primarily directed to one surface immunogen of *T. vaginalis* was demonstrated. The P230 immunogen was previously studied with a MAb, and the protein undergoes a property called epitope phenotypic variation.⁷ This was based on the variable accessibility of the epitope to Ab binding, even though the molecule resides on the parasite surface at all times. Not surprisingly, flow cytometric analysis with the MAb (fig 3) revealed isolates such as T032 that were either

uniformly fluorescent trichomonads or heterogeneous isolates such as T040, comprised of fluorescent and nonfluorescent organisms. In both isolates pooled VW Ab to P230 (fig 3) detected only some trichomonads, even in isolate T032, in which all parasites were immunoreactive with the MAb. The different patterns obtained for isolate T032 with the MAb and VWs are indicative of recognition of unique epitopes with the respective antibodies. Further, the immunoreactivity with only some organisms may also suggest a very restricted type of Ab response to P230 by infected women.

The duration in treated women of the vaginal Ab response to P230 was especially noteworthy (fig 4). Even after elimination of the parasites from the host, an elevation in the titre of Ab, based on increased band intensity of autoradiograms, was evident. This vaginal Ab to P230 appears ineffective and without value in host protection, since the immunoreactive vaginal Ab was not cytolytic to trichomonads. Complement, which is only made available to the site of infection during menstruation, added to the parasites with bound vaginal Ab or the MAb, was also without effect in killing trichomonads. Regardless of whether the anti-P230 Ab was cytolytic, the presence of a subpopulation of *T. vaginalis* organisms with epitopes inaccessible to vaginal Ab binding (fig 4) would insure the survival of and possibly select for only non-immunoreactive parasites. Whatever the unique features of the P230 immunogen and the parasite surface, the immune evasion strategies of *T. vaginalis* are illustrated by the fact that all vaginal Ab is restricted toward this single surface protein, which undergoes epitope phenotypic variation.⁷

This study was supported by Public Health Service grant AI-22380 from the National Institute of Allergy and Infectious Diseases and by a grant from the South Texas Vaccine Development Center. The excellent secretarial assistance of Diana Hinojosa and the technical help of Lorraine Kasmala are especially acknowledged. The help of Dr John Kung and Charles Thomas during the flow cytometric analysis is gratefully acknowledged.

Address for correspondence: Dr John F Alderete, Department of Microbiology, UTHSCSA, 7703 Floyd Curl Drive, San Antonio, Texas 78284-7758, USA

- 1 Rein MF, Chapel TA. Trichomoniasis, candidiasis, and the minor venereal diseases. *Clin Obstet Gynecol* 1975;18:73-88.
- 2 Alderete JF. Enzyme-linked immunosorbent assay for detection of antibody to *Trichomonas vaginalis*: Use of whole cells and aqueous extract as antigen. *Br J Venereal Dis* 1984;60:164-70.
- 3 Street DA, Taylor-Robinson D, Ackers JP, Hanna NF, McMillan A. Evaluation of an enzyme-linked immunosorbent assay for the detection of antibody to *Trichomonas vaginalis* in sera and vaginal secretions. *Br J Venereal Dis* 1982;58:330-3.

- 4 Su KE. Antibody to *Trichomonas vaginalis* in human cervico-vaginal secretions. *Infect Immun* 1982;37:852-7.
- 5 Lossick JG. Treatment of sexually transmitted vaginosis/vaginitis. *Rev Infect Dis* 1990;12(Suppl 6):5665-81.
- 6 Alderete JF, Demeš P, Gombošova A, et al. Specific parasitism of purified vaginal epithelial cells by *Trichomonas vaginalis*. *Infect Immun* 1988;56:2558-62.
- 7 Alderete JF, Demeš P, Gombošova A, et al. Phenotypes and protein/epitope phenotypic variation among fresh isolates of *Trichomonas vaginalis*. *Infect Immun* 1987;55:1037-41.
- 8 Alderete JF, Kasmala L. Monoclonal antibody to a major glycoprotein immunogen mediates differential complement-independent lysis of *Trichomonas vaginalis*. *Infect Immun* 1986;53:697-9.
- 9 Alderete JF, Neale KA. Relatedness of major immunogen among all *Trichomonas vaginalis* isolates. *Infect Immun* 1989;56:1849-53.
- 10 Alderete JF, Suprun-Brown L, Kasmala L. Monoclonal antibody to a major surface glycoprotein immunogen differentiates isolates and subpopulations of *Trichomonas vaginalis*. *Infect Immun* 1986;52:70-5.
- 11 Diamond LS. The establishment of various trichomonads of animals and man in axenic cultures. *J Parasitol* 1957;43:488-90.
- 12 Lockwood BC, North MJ, Coombs GH. The release of hydrolases from *Trichomonas vaginalis* and *Tritrichomonas foetus*. *Molec Biochem Parasitol* 1988;30:135-42.
- 13 Neale KA, Alderete JF. Analysis of the proteinases of representative *Trichomonas vaginalis* isolates. *Infect Immun* 1990;58:157-62.

Accepted for publication 7 January 1991.